

# Selectin Biosciences Inc.

## Endoglycosidase F3

Endoglycosidase F3 [Endo- $\beta$ -N-acetylglucosaminidase F3, EC 3.2.1.96] cleaves asparagine-linked biantennary and triantennary complex, oligosaccharides depending on the state of core fucosylation and peptide linkage (see Figure 1). It cleaves between the two N-acetylglucosamine residues in the diacetylchitobiose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. In contrast, PNGase F removes the oligosaccharide intact. There is no activity on oligomannose and hybrid molecules.

Endoglycosidase F3 will also cleave trimannosylchitobiose core structures (see Figure 1). This activity was previously attributed only to Endoglycosidase D from *Streptococcus* (formerly *Diplococcus*) *pneumoniae*.

Endoglycosidase F3 is less sensitive to protein conformation than PNGase F and is therefore more suitable for deglycosylation of native proteins. However for optimal results, denaturation of the glycoprotein is recommended.

Endoglycosidase F3 is isolated from a strain of *E. coli* expressing a cloned gene from *Elizabethkingia miricola*. The recombinant protein is not glycosylated. This alteration may result in properties that differ from the natively-derived protein.

**Product Code:** GE 49

## Specifications

**Activity:**  $\geq 25$  U/mg,  $\geq 5$  U/mL

**Storage:** Store at 4°C. Do not freeze.

**Formulation:** The enzyme is provided as a sterile-filtered solution in 20 mM Tris-HCl pH 7.5.

**Stability:** Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

## Assay

One unit of Endoglycosidase F3 activity is defined as the amount of enzyme required to catalyze the release of 1  $\mu$ mole of N-linked oligosaccharides from porcine fibrinogen glycopeptides in 1 minute at 37°C, pH 4.5.

## Product Description

**Molecular weight:** 30,000 Daltons

**Purity:** Each lot of Endoglycosidase F3 is tested for contaminating activities by incubating the enzyme for 24 hours at 37°C with 2  $\mu$ L of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

**Specificity:** Asparagine-linked or free bi- and triantennary oligosaccharides depending on core fucosylation and peptide linkage.

## Reagents

- 5X Reaction buffer 4.5 - 250 mM sodium acetate pH 4.5.
- Denaturation Solution – w/v sodium lauryl sulfate, 1 M  $\beta$ -mercaptoethanol

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- Triton X-100 solution\*, 15% v/v Triton X-100

## Suggestions for Use

### Procedure for Deglycosylation

1. Add up to 200 µg of glycoprotein to Eppendorf tube
2. Add deionized water to a total of 33 µL
3. Add 10 µL 5X Reaction Buffer, 4.5
4. Add 2.5 µl of Denaturation solution. Heat at 90°C for 10 minutes
5. Cool to room temperature and add 2.5 µL Triton X-100 solution
6. Add 2 µL of Endoglycosidase F3. Incubate 1 hour or more at 37°C
7. Monitor cleavage by SDS-PAGE

For digestion of native proteins, add water to a total volume of 38 µL and omit steps 4 and 5. Increase incubation time appropriately.

## References

1. Anumula, K.R. Endo beta-N-acetylglucosaminidase F cleavage specificity with peptide free oligosaccharides. **J Mol Recognit** **6**:139-145 (1993).
2. Maley F., R. B. Trimble, A. L. Tarentino and T. H. Plummer Jr. Characterization of glycoproteins and their associated oligosaccharides through the use of

endoglycosidases. **Anal Biochem** **180**:195-204 (1989).

3. Plummer, T. H. Jr, A. W. Phelan and A. L. Tarentino. Porcine fibrinogen glycopeptides: substrates for detecting endo-beta-N-acetylglucosaminidases F2 and F3. **Anal Biochem** **235**:98-101 (1996).
4. Reddy A., B. G. Grimwood, T. H. Plummer Jr and A. L. Tarentino. High-level expression of the Endo-beta-N-acetylglucosaminidase F2 gene in E.coli: one step purification to homogeneity. **Glycobiology** **8**:633-636 (1998).
5. Tarentino, A. L., C. M. Gomez and T. H. Plummer Jr. Deglycosylation of Asparagine-Linked Glycans by Peptide:N-Glycosidase F. **Biochemistry** **24**:4665-4671 (1985).
6. Tarentino A. L., G. Quinones, W. P. Schrader, L. M. Changchien and T. H. Plummer Jr. Multiple endoglycosidase (Endo) F activities expressed by *Flavobacterium meningosepticum*. Endo F1: molecular cloning, primary sequence, and structural relationship to Endo H. **J Biol Chem** **267**:3868-3872 (1992).
7. Tarentino A. L., G. Quinones, L. M. Changchien, and T. H. Plummer Jr. Multiple endoglycosidase F activities expressed by *Flavobacterium meningosepticum* endoglycosidases F2 and F3: Molecular cloning, primary sequence, and enzyme expression. **J Biol Chem** **268**(13):9702-9708 (1993).

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8. Tarentino A. L. and T. H. Plummer Jr.  
Substrate specificity of *Flavobacterium meningosepticum*: Endo F2 and endo F3: purity is the name of the game. **Glycobiology** **4**:771-773 (1994).
9. Tarentino, A. L. and T. H. Plummer Jr.  
Enzymatic deglycosylation of asparagine-linked glycans: purification, properties and specificity of oligosaccharide-cleaving enzymes from *Flavobacterium meningosepticum*. **Methods in Enzymology** **230**:44-57 (1994).
10. Tarentino A. L., G. Quinones and T. H. Plummer Jr. Overexpression and purification of non-glycosylated recombinant endo-beta-N-acetylglucosaminidase F3. **Glycobiology** **5**:599-601 (1995).
11. Trimble, R. B. and A. L. Tarentino.  
Identification of Distinct Endoglycosidase (Endo) Activities in *Flavobacterium meningosepticum*: Endo F1, Endo F2 and Endo F3. **J. Biol Chem** **266**:1646-1651 (1991).

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This product is intended for in vitro research only.

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## Figure 1 - Cleavage of oligosaccharides by Endo F3

Man - Mannose; Gal - Galactose; Fuc - Fucose; GlcNAc - N-acetylglucosamine; NeuAc - N-acetylneuraminic acid;  
R - Peptide linkage required

